Developmental Cell *15*

Supplemental Data

A Repressor Complex Governs the Integration of Flowering Signals in *Arabidopsis*

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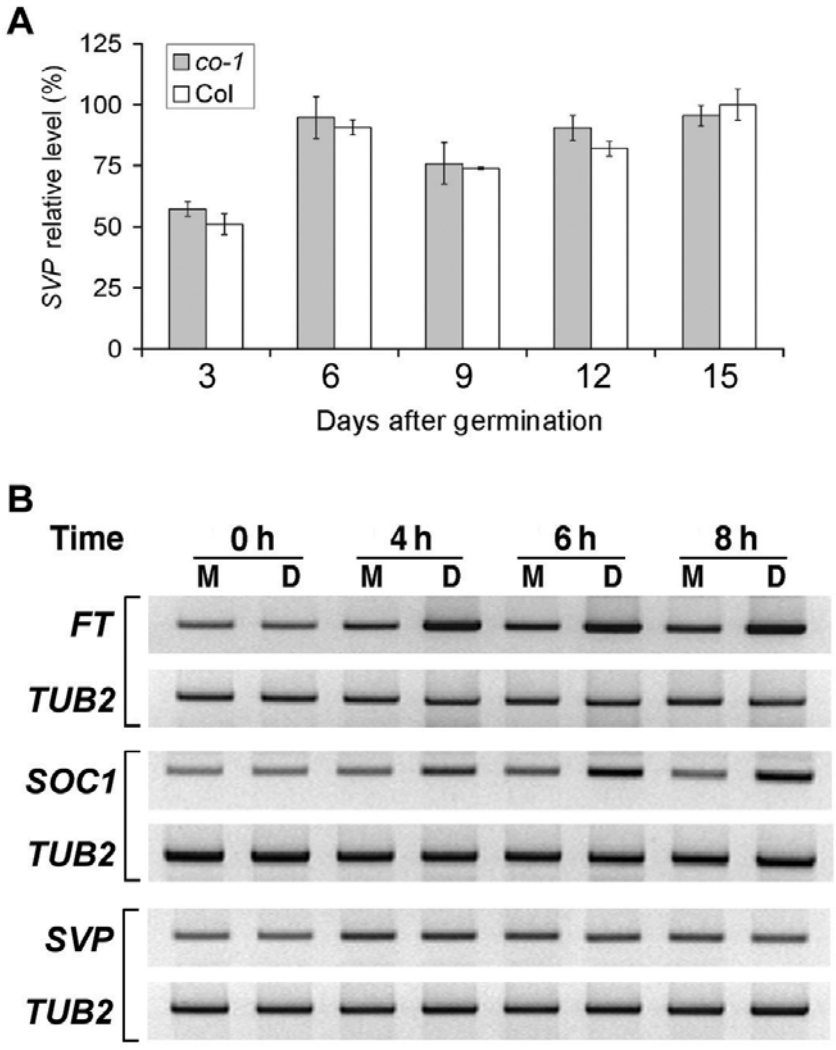

Figure S1. *SVP* Expression is not Affected by *CO* Activity

(A) Temporal expression of *SVP* in Col wild-type and *co-1* seedlings grown in LDs. Transcript levels were determined by quantitative real-time PCR analyses of three independently collected samples. Results were normalized against the expression of *TUB2*. Error bars indicate SD. Compared with *SVP* expression in wild-type plants, its expression remains almost unchanged in *co-1*.
(B) Time course expression of *FT*, *SOC1*, and *SVP* in 11-day-old *35S::CO-GR co-2* grown on MS medium (3% sucrose and 0.03% phytagel) under LDs. Seedlings were mock-treated (M) or treated with 10 μM dexamethasone (D). Transcript levels were determined by semi-quantitative RT-PCR analyses of three independently collected samples. The representative profiles are shown here. Induced *CO* activity can obviously upregulate *FT* and *SOC1*, but has no effect on *SVP* expression.

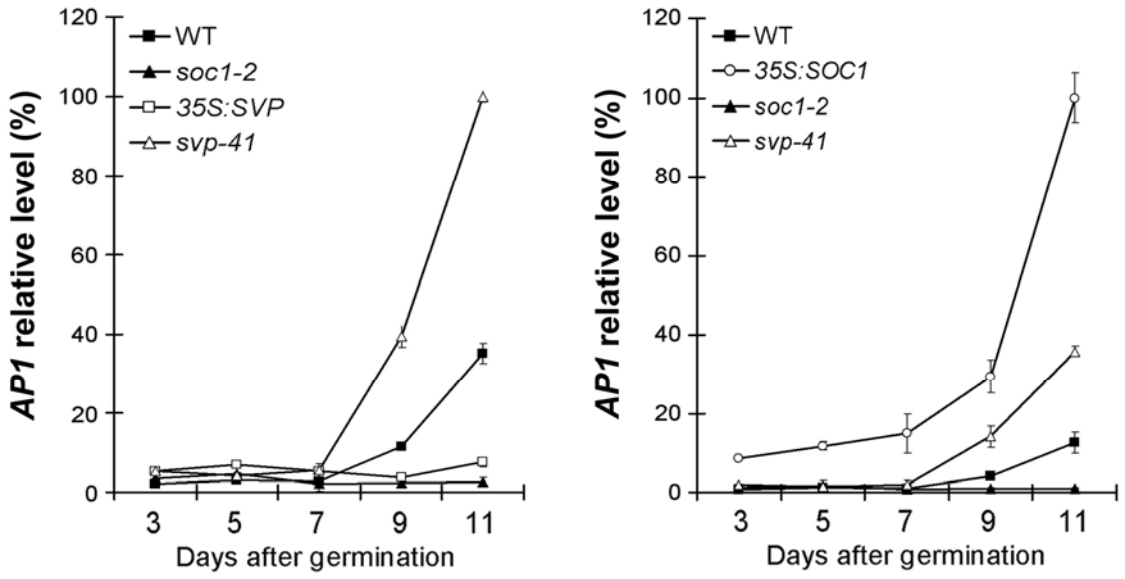

Figure S2. Temporal Expression of *AP1* in Developing Seedlings with Various Genetic Backgrounds in LDs
Transcript levels were determined by quantitative real-time PCR analyses of three independently collected samples. Results were normalized against the expression of *TUB2*. Error bars indicate SD. The floral transition in wild-type plants is marked by the significantly increased expression of a floral meristem identity gene *APETALA1* (*AP1*) at 9 days after germination.

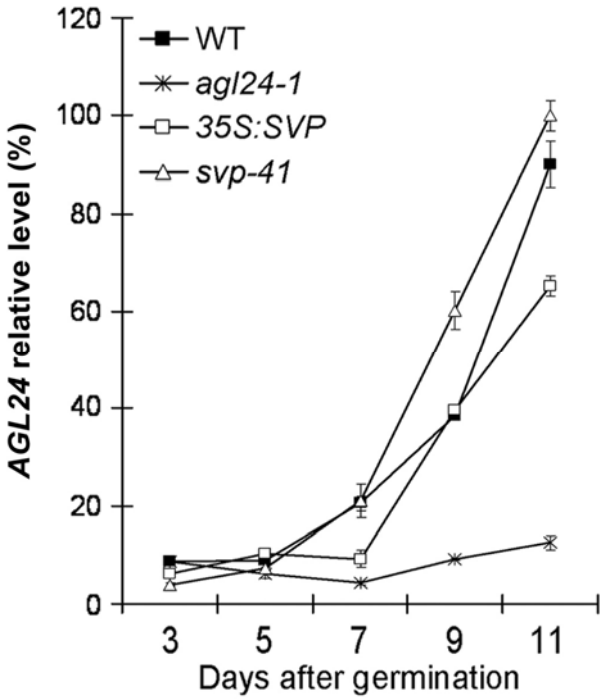

Figure S3. Temporal Expression of *AGL24* in *svp-41* and *35S:SVP* Seedlings Grown in LDs

Transcript levels were determined by quantitative real-time PCR analyses of three independently collected samples. Results were normalized against the expression of *TUB2*. Error bars indicate SD.

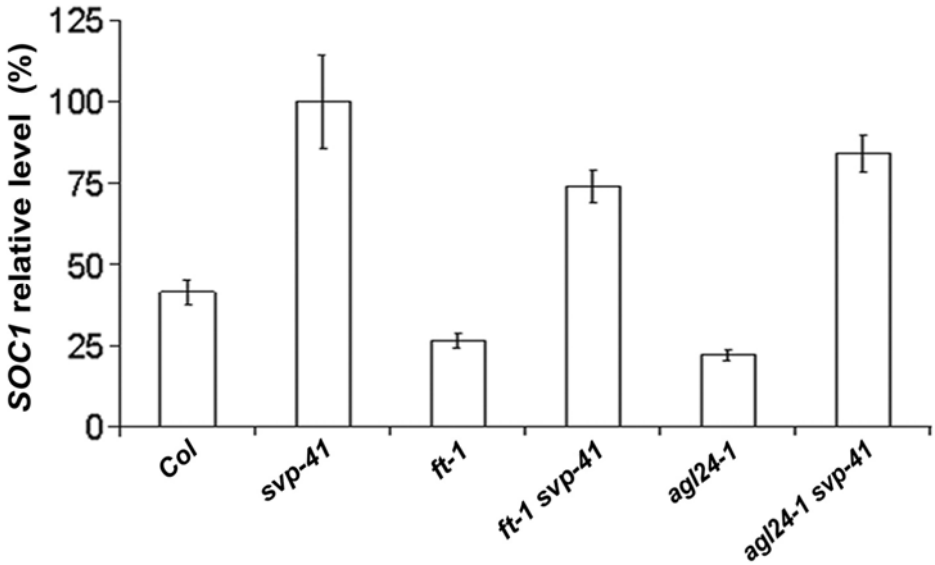

Figure S4. Loss of *SVP* Function Derepresses *SOC1* Expression Largely Independently of *FT* and *AGL24*

SOC1 expression in 9-day-old seedlings with various genetic backgrounds in LDs was measured by quantitative real-time PCR. *TUB2* was used for normalization. Error bars indicate SD.

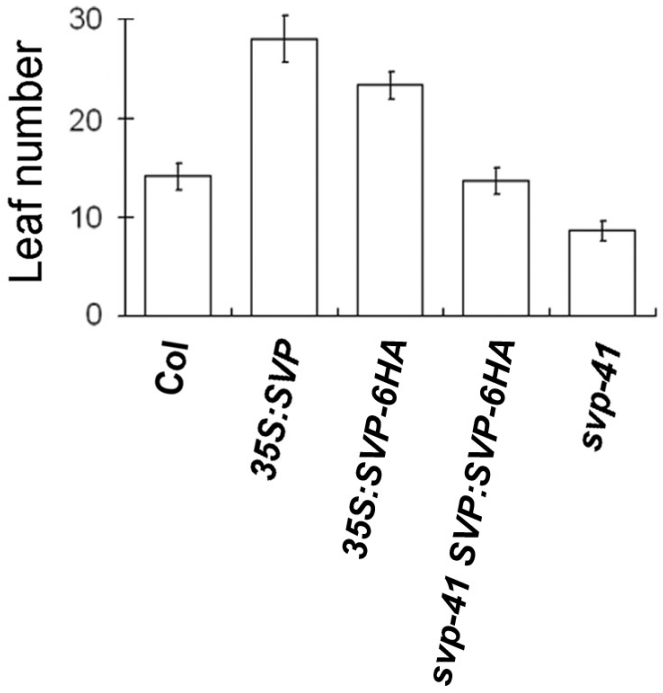

Figure S5. Generation of Functional *35S:SVP-6HA* and *SVP:SVP-6HA* Transgenic Lines

Flowering time of generated transgenic lines in LDs was compared by calculating the number of total leaves. Values representing the mean ±SD were scored from at least 20 plants of each genotype. *35S:SVP-6HA* and *35S:SVP* plants show late flowering as compared with wild-type plants. *svp-41* mutants show early flowering, while *svp-41 SVP:SVP-6HA* plants exhibit comparable flowering time as wild-type plants, indicating that SVP-6HA protein retains the biological function as endogenous SVP.

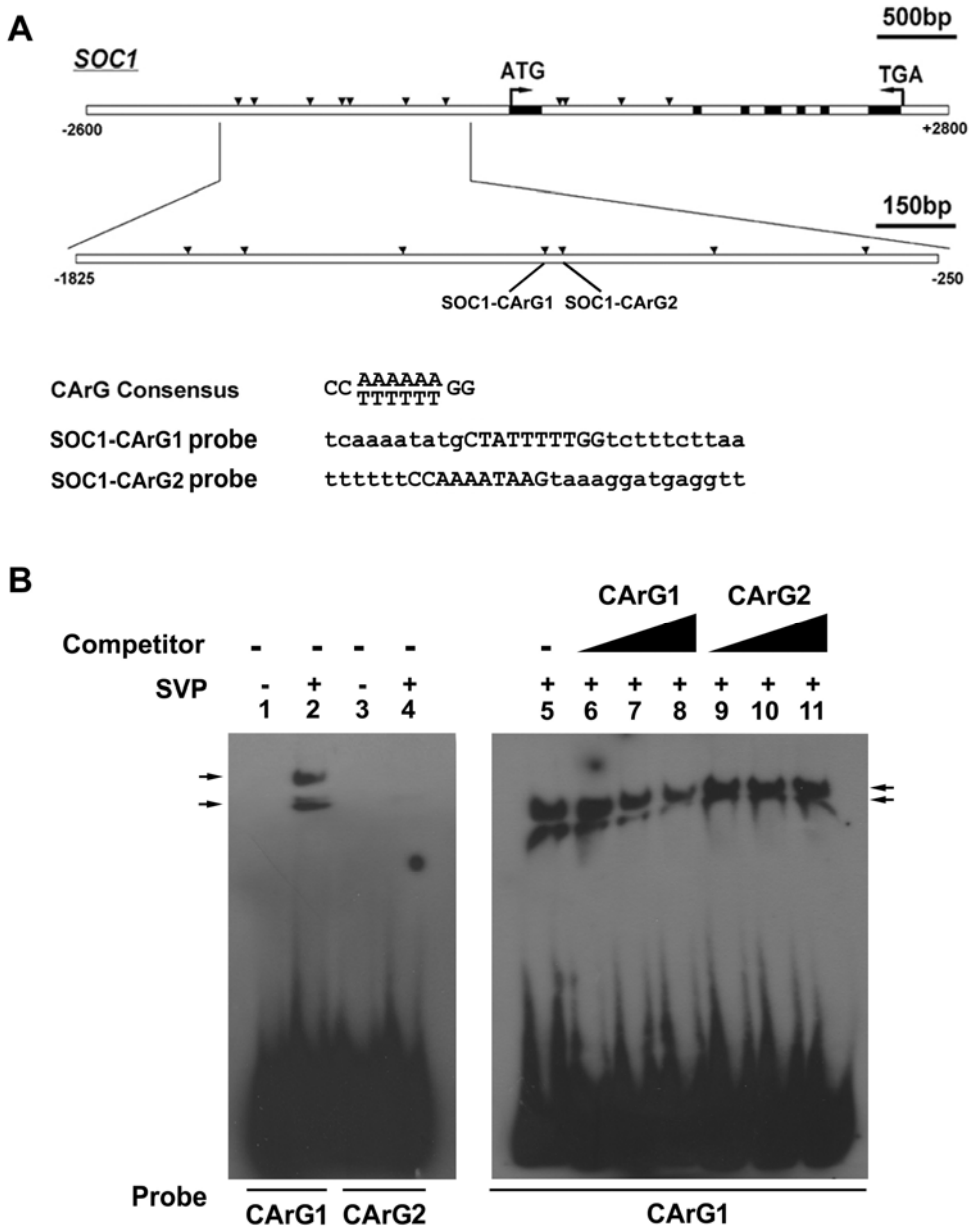

Figure S6. Specific Binding of SVP Protein to the SOC1-CArG1 Sequence
(A) List of two probes containing putative MADS-box binding sites (CArG boxes) used for gel shift assays.
(B) Preferential binding of 6His-SVP protein to a 30 bp fragment containing the SOC1-CArG1 sequence (arrows). 6His-SVP protein was incubated with either SOC1-CArG1 or SOC1-CArG2 probe as indicated below the panel. The presence of 6His-SVP protein and unlabelled competitor DNA is indicated above the panels. Lanes 1 and 3, no protein and no competitor DNA; lanes 2, 4 and 5, 6His-SVP protein and no competitor DNA; lanes 6, 7 and 8, SOC1-CArG1 fragment as competitor DNA; lanes 9, 10, and 11, SOC1-CArG2 fragment as competitor DNA. Non-labeled DNA in molar excess was used as competitor in lanes 6 and 9 (50-fold), lanes 7 and 10 (200-fold), and lanes 8 and 11 (800-fold).

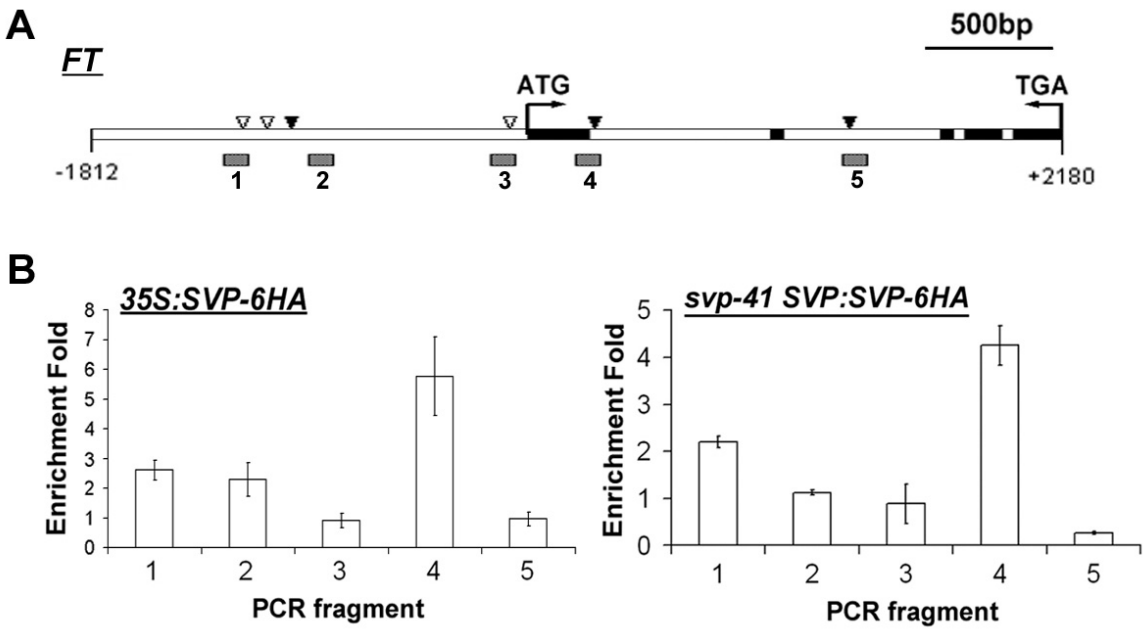

Figure S7. SVP is Associated with the *FT* Genomic Region

(A) Schematic diagram of the *FT* genomic region. Exons are represented by black boxes, while introns and upstream regions are represented by white boxes. Bent arrows denote translation start sites and stop codons. Filled arrowheads indicate the sites containing either single mismatch or perfect match with the consensus binding sequence (CArG box) of MADS-domain proteins, while open arrowheads indicate the CArG sites containing two mismatches. Five DNA fragments near the above CArG sites in the *FT* genomic region were examined by ChIP enrichment test as shown in (B).

(B) ChIP enrichment test shows the binding of SVP-6HA to the region near the fragment 4. Seven-day-old seedlings of *35S:SVP-6HA* and *svp-41 SVP:SVP-6HA* were harvested for ChIP analysis. Relative enrichment of each fragment was calculated first by normalizing the amount of a target DNA fragment against a genomic fragment of *ACTIN*, and then by normalizing the value for transgenic plants against the value for wild-type as a negative control.

Table S1. Primer Pairs Used for ChIP Assays

Primer Pairs Used for ChIP Assay of SVP Binding on the *SOC1* Sequence

Primer	Sequence
1	5′-TATATCGGGAGGAGGACCACAC-3′
	5′-ATCCATACAGATTTTCGGACCT-3′
2	5′-GAGGCTAGTACAGAGACAATGG-3′
	5′-GACCAAAAATAGCAAATGCCTC-3′
3	5′-TCTCGTACCTATATGCCCCCACT-3′
	5′-TTTATCTGTTGGGATGGAAAGA-3′
4	5′-AGTTGGATGGAAATGCCTGTCA-3′
	5′-TTACAAGTGGGGGCATATAGGT-3′
5	5′-TGGACGCTTGAAACCTCATCCT-3′
	5′-GGGAGGGAAAAAGATGTGTATG-3′
6	5′-GCAAAAGAAGTAGCTTTCCTCG-3′
	5′-AGCAGAGAGAGAAGAGACGAGTG-3′
7	5′-AAAAACCTAACCAGGAGGAAGC-3′
	5′-CTTCTTCTCCCTCCAGTAATGC-3′
8	5′-GGATGCAACCTCCTTTCATGAG-3
	5′-ATATGGGTTTGGTTTCATTTGG-3′
9	5′-ATCACATCTCTTTGACGTTTGCTT-3′
	5′-GCCCTAATTTTGCAGAAACCAA-3′
10	5′-CTTTTGGTTTGAACTAATCTTTGTCTTG-3′
	5′-AATGAGCATGAAATGAAGCATGA-3′
11	5′-TGTTTCAGACATTTGGTCCATTTG-3′
	5′-AGTCTTGTACTTTTTCCCCCTATTTTAG-3′
ACTIN	5′-CGTTTCGCTTTCCTTAGTGTTAGCT-3′
	5′-AGCGAACGGATCTAGAGACTCACCTTG-3′

Primer Pairs Used for ChIP Assay of SVP Binding on the *FT* Sequence

Primer	Sequence
1	5′-GCAATGTCAAAAAGAAAATCTCTCAA-3′
	5′-TGCACGACCAGGATAATTGG-3′

2	5’-CGTATTTGAGTTCGGACATTGG-3’
	5’-TCAAACATGTAGAATGAAGGCAGTTA-3’
3	5’-TGATTTCACCGACCCGAGTT-3’
	5’-AGGCATGAACCCTCTACACATATTTA-3’
4	5’-CAAGAGTTGAGATTGGTGGAGAAG-3’
	5’-CAAAAGGGAGTTCAAGTGAAAGAAC-3’
5	5’-CATCAATTTGTCTCCCAAAAAAGC-3’
	5’-GCGATCAGTAAAATACACAGACATACATAA-3’
ACTIN	5’-CGTTTCGCTTTCCTTAGTGTTAGCT-3’
	5’-AGCGAACGGATCTAGAGACTCACCTTG-3’

Table S2. Primer Pairs Used for Gene Expression Analysis

Primer pairs used for quantitative real-time PCR

Gene	Sequence
AGL24	5′-GAGGCTTTGGAGACAGAGTCGGTGA-3′
	5′-AGATGGAAGCCCAAGCTTCAGGGAA-3′
SOC1	5′-AGCTGCAGAAAACGAGAAGCTCTCTG-3′
	5′-GGGCTACTCTCTTCATCACCTCTTCC-3′
AP1	5′-CATGGGTGGTCTGTATCAAGAAGAT-3′
	5′-CATGCGGCGAAGCAGCCAAGGTT-3′
SVP	5′-CAAGGACTTGACATTGAAGAGCTTCA-3′
	5′-CTGATCTCACTCATAATCTTGTCAC-3′
FT	5′-CTTGGCAGGCAAACAGTGTATGCAC-3′
	5′-GCCACTCTCCCTCTGACAATTGTAGA-3′
TUB2	5′-ATCCGTGAAGAGTACCCAGAT-3′
	5′-AAGAACCATGCACTCATCAGC-3′

Primer pairs used for semi-quantitative PCR

Gene Amplified	Primer Pair
SOC1	5′-GAAGATATGGTGAGGGGCAAAACTC-3
	5′-GGGCTACTCTCTTCATCACCTCTTCC-3′
FT	5′-CCCTGCTACAACTGGAACAACCTTT-3′
	5′-TAGGCATCATCACCGTTCGTTACTC-3′
SVP	5′-GTGACAAGATTATGAGTGAGATCAG-3′
	5′-GAATTCACTACTTAGACATTGTCTC-3′
TUB2	5′-ATCCGTGAAGAGTACCCAGAT-3′
	5′-TCACCTTCTTCATCCGCAGTT-3′